New opportunities in animal breeding and production — an introductory remark

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Abstract

New opportunities in animal breeding and production will be offered by methods for embryo production and the introduction of precise genetic changes in livestock species. A review of current procedures shows that they have been improved during the last 4 years, but still have significant limitations. In the light of experience over the past 50 years it seems likely that these methods will contribute to animal health and productivity, but at some unknown time in the future and in ways that cannot be predicted precisely. When looking to the future, delegates to this meeting should take pride in what science continues to contribute. © 2000 Published by Elsevier Science B.V. All rights reserved.

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1. Introduction

Methods for embryo production and for precise genetic modification of livestock will offer new opportunities in animal breeding and production. Progress towards those goals has continued during the 4 years since the last of these meetings. The purpose of this analysis is to summarise progress and the present limitations before considering the implications for the future. We are concerned only with cattle, sheep, pigs and goats.

2. Embryo production in vitro

There appears to be a contrast between ruminants and the pig in the effectiveness of current procedures. Methods for in vitro maturation (IVM) of oocytes, fertilisation and

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Embryo culture have been used extensively in cattle (Leibfried-Rutledge et al., 1989) and sheep (Walker et al., 1992). The methods are also being used in cattle breeding schemes after aspiration of oocytes from selected females and to produce calves from casualties. By contrast, despite a considerable research effort and the introduction of several novel steps, only a very small proportion of pig oocytes developed to offspring (Prather and Day, 1998).

In the past, development was perturbed in some ruminant offspring produced in vitro (reviewed by Young et al., 1998). The most obvious symptom is an increase in birthweight, to an extent that may be sufficient to prejudice survival of mother and offspring. Although pregnancy is extended this is not the cause of the large size as differences arise early in pregnancy (see Young et al., 1998). Not only are some offsprings unusually large, but the weight of the liver, kidney and heart is sometimes disproportionately large (Sinclair et al., 1999) and there is an increased incidence of developmental abnormalities. Expression of imprinted genes is being studied in the light of the similarities between the Large Offspring syndrome in livestock, children with congenital abnormalities such as Beckwith–Wiedemann syndrome and mice with targeted changes in their genes (see Young et al., 1998).

Embryo transfer can be used much more efficiently if there is a method for embryo storage. Otherwise a large group of potential recipients must be available and these increase the cost very markedly. While cryopreservation of cattle and sheep embryos has been achieved and is widely used (Rall, 1992), in the other species, it is still in the developmental stage. In particular, pig embryos are very sensitive to chilling injury. Recent experiments show that useful survival of pig embryos could be obtained if their lipid content was reduced before vitrification (Dobrinsky et al., 1999).

Further research is needed to provide methods for embryo production and storage in pigs and to make those in ruminants more efficient. After culture, the embryos must also be capable of normal development to term.

3. Nuclear transfer

One of the significant advances of the last 4 years has been the widespread use of nuclear transfer from somatic cells. This advance depended upon coordination of cell cycle in donor and recipient cells (reviewed by Campbell et al., 1996a). Several laboratories working independently have now produced offspring in three species (sheep, cattle and goat), however, at the time of writing in 1999 there are no reports of piglets derived from somatic cells. These result in ruminants followed the first induction of quiescence in donor cells (Campbell et al., 1996b) and in most cases the donor cells were either expected to be quiescent or were induced to leave the growth cell cycle and become quiescent by serum starvation. In one claimed exception (Cibelli et al., 1998) the authors provided no evidence to support their claim that the cells were in G1 phase of the cycle (see Wilmut and Campbell, 1998).

An alternative approach has been pioneered by Chesne et al., (1993). They discovered that, after aging of the bovine oocyte at reduced temperatures, the level of MPF is reduced. As a result, nuclear envelope breakdown does not occur after nuclear transfer.
In these circumstances, it is expected that the transferred nucleus will itself determine whether DNA replication takes place. Using this approach to cell cycle coordination, calves have been produced without synchronisation of the donor cell cycle (Vignon et al., 1998).

Although nuclear transfer has proved to be repeatable, present procedures all share significant limitations. Only 1–2% of reconstructed embryos survive to become live offspring. In part, this reflects a far greater than normal pre-natal mortality that, unlike in normal pregnancies is spread throughout gestation. Similarly, greater perinatal mortality is associated with congenital abnormalities and the symptoms of large offspring syndrome described previously. In one case, an apparently normal cloned, bull calf died at day 50 after birth because of lymphoid hypoplasia (Renard et al., 1999). Further research is essential to understand the mechanisms of reprogramming gene expression in the transferred nucleus and the causes of failure. In time this will also increase the proportion of cloned embryos able to develop to term.

Application of nuclear transfer in animal selection schemes at present is not practicable because of the low efficiency (Dematawewa and Berger 1998) in particular the late pre- and perinatal loss. However, there is the potential to significantly enhance the dissemination of genetic improvement from selection herds at some time in the future. Alternatively, there may be a sufficient difference in productivity between animals in the same herd for the owner to consider paying to have copies made of selected animals. Certainly, in that situation the owner knows that the estimate of value has been made in the environment in which the clone will perform. Nuclear transfer from somatic cells will allow the introduction of precise genetic change in livestock in addition to the beneficial effects in selection schemes.

4. Genetic modification by nuclear transfer

The ability to produce offspring by nuclear transfer from cultured cells has provided new opportunities for genetic modification. Both gene addition (Schnieke et al., 1997) and precise genetic modification have been achieved (personal communication). In the past, genetic modification of livestock could only be achieved by injection of several hundred copies of a gene into a nucleus in the early embryos (Eyestone, 1998). The use of cultured cells has several potential advantages in comparison to the method of direct injection. First, it is possible to use DNA sequencing to confirm that the desired change has been introduced before nuclear transfer to produce offspring. Hence, they will all have the desired genetic change. Secondly, it is possible to use gene-targeting techniques to make precise genetic change in endogenous genes, rather than merely add genes. This creates a whole range of new opportunities when genes are switched off or given a different function. Finally, several copies of the modified offspring can be obtained and the effect of the genetic change can be measured accurately by comparing the modified offspring with offspring from the same cells that had not been genetically modified.

At present genetic modification is planned for biomedical applications, such as the production of proteins for the treatment of human diseases (Garner and Coleman, 1998) or of pig organs for transplantation (White and Lanford 1998). In part this probably
reflects the greater sums of money available for investment in these areas. However, recent reviews of projects for agricultural applications identify only a small number of candidate genes for modification (Pursel, 1998). As genes are identified by livestock gene mapping projects and their function studied by genetic modification, then more potential applications for genetic modification will probably become apparent.

5. Implications for the future

In order to have the present advances in context we should consider those achieved during the 50 years since this series of meetings was initiated. The progression over this period has been rapid, but many basic problems remain unsolved. Some applications that initially were heralded by new discoveries have yet to be fully realised.

6. Artificial insemination (AI)

Of all technologies to be discovered, AI is the one that has, so far, made the greatest impact on animal production. During the past 50 years, use of AI has contributed much more in both the control of disease and genetic improvement. Addition of penicillin to semen in eliminated Campylobacter (Vibrio) from semen. Campylobacter had been a major cause of infertility in cattle, but now AI offered a method of control. The discovery of methods to freeze cattle semen by Polge et al. (1949) in the late 1940s revolutionised AI in cattle that until then had depended upon a chilled product with a very limited life. Frozen semen could now be stored indefinitely used widely and even exported easily. Later, an understanding of the reproductive endocrinology of the female led to the development of methods for the synchronisation of oestrus by administration of prostaglandins or progestagens. This extended the practical use of AI to dairy heifers and to beef cattle.

There are many situations in which it would be commercially very useful to produce calves of a specified sex. Recently there have been considerable steps forward in the separation of X and Y bearing spermatozoa (as we will hear later at this meeting), allowing pre-selection of gender prior to insemination (Johnson 1996). Over 90% of offspring are of the predicted sex and this technology has been shown to be effective in several species. Present methods have a significant limitation in that one cell sorter is only able to produce a very small number of sperm doses in a day, although fewer sperm are required in they are deposited further along the uterine horn (Seidel et al., 1998).

While methods for AI were expected to eliminate venereal diseases, they have also had profound effects upon genetic progress. Over the lifetime of these meetings, milk production of our average milk-cow has more than doubled, substantially because of the use of frozen and thawed semen from progeny tested bulls.

Despite all the progress with AI, there are still many basic hurdles yet to be overcome. Although freezing of cattle semen has been a great success we have failed to achieve similar results with other farm species. When sheep semen is frozen and thawed reliable conception rates can only be achieved by surgical insemination (Haresign 1992).
Similarly, boar sperm fertility is reduced by freezing and thawing (Polge et al., 1970). The impact of AI in cattle reflects in part the ability to deposit the sperm beyond one of the main barriers to sperm transport in that species. By contrast, in sheep, sperm can only be deposited in the uterus easily by surgical intervention. In the pig, the cervix is not a barrier to sperm.

7. Embryo transfer

After the success of AI, research concentrated upon the development of methods for embryo transfer, to offer in female reproduction the opportunity for genetic selection that had become possible in the male. In addition to the perceived potential for breed improvement by embryo transfer, in the UK, much was written of the potential for production of beef calves from dairy females.

Several technical developments were required for practical embryo transfer in livestock, including methods for the induction of superovulation and synchronisation of oestrus for the species involved and those for embryo recovery, storage and transfer. Without the means to superovulate, a donor the technique would have been of little value. Success of transfer was shown to depend critically on synchrony of oestrus in donor and recipient females (see Rowson et al., 1972). The development of both non-surgical embryo recovery and transfer in cattle has allowed the technique to be used more widely in the field. Similar technical progress in other species has not followed, although efficiencies in some of the surgical techniques have been achieved (McKelvey et al., 1986). Sexing of embryos prior to transfer is now possible using polymerase chain reaction (PCR). Although accuracy is 95% and 50–70% of biopsied grade 1 embryos establish pregnancies, the technique is not used as a routine procedure (Bredbacka 1998). One economic factor is that usually half the embryos are discarded because the breeder only wants calves of one sex. This has the effect of doubling the charge that must be made for each embryo transferred.

Initially embryo transfer was mainly used for the production of high merit pedigree animals which were in short supply, but increasingly the technique is being used for evaluation and breed improvement by carrying out multiple ovulation and embryo transfer (MOET schemes). Embryo transfer in cattle has flourished because of the development of reliable synchronisation and superovulation programmes, non-surgical embryo recovery and transfer techniques and the ability to cryopreserve embryos. However, embryo transfer is used far less than AI. A comparison of costs and benefits shows that often AI offers more advantages for a smaller cost. The full utilisation of embryo transfer in other domestic species lags well behind that of cattle because some or all of these enabling technologies are still not in place.

8. Embryo manipulation

The facility to split embryos generating identical twins was developed 2 decades ago and embryo splitting was offered as a commercial service. Whereas direct transfer of
embryos yielded around 60 calves per 100 embryos, transfer of split embryos typically produced 105 calves per 100 embryos. The limitation of only being able to produce two identical animals was lifted when embryo nuclear transfer was successfully achieved using blastomeres (Willadson et al., 1991). The first commercial use of nuclear transfer was short lived partly because of the low efficiency and the birth of unusually large offspring (Wilson et al., 1995). This early work on embryo manipulation led eventually to the nuclear transfer technology already discussed that produced Dolly and other adult clones.

Overall, it seems that practical application of embryo related techniques has been less than anticipated, despite considerable technical progress.

9. Animal health

Animal health has taken huge steps forward during last 50 years as a result of progress in many areas, including the development of new pharmaceuticals and vaccines. Table 1, which was adapted from Dunlop and Williams (1996), illustrates well the progress in disease control achieved over the last 50 years in the USA.

Table 1

<table>
<thead>
<tr>
<th>Year</th>
<th>Event</th>
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<tbody>
<tr>
<td>1951</td>
<td>New test differentiate foot-and-mouth disease, vesicular stomatitis and vesicular exanthema</td>
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<tr>
<td>1952</td>
<td>Cause of bovine hyperkeratosis is discovered</td>
</tr>
<tr>
<td>1953</td>
<td>Vibriosis is identified as a cause of bovine infertility</td>
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<tr>
<td>1954</td>
<td>Research of foreign animal diseases begins on Plum Island</td>
</tr>
<tr>
<td>1958</td>
<td>Virus that causes bovine rhinotracheitis is discovered</td>
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<tr>
<td>1959</td>
<td>A virus involved with the bovine shipping fever complex is isolated</td>
</tr>
<tr>
<td>1959</td>
<td>Screwworm flies are eradicated from the American Southwest</td>
</tr>
<tr>
<td>1959</td>
<td>Vesicular exanthema is eradicated</td>
</tr>
<tr>
<td>1960</td>
<td>Domestic animal health research and diagnostics are consolidated at the National Animal Disease Laboratory (now the National Animal Disease Control Centre) in Ames, IA</td>
</tr>
<tr>
<td>1965</td>
<td>Rapid test for hog cholera is developed</td>
</tr>
<tr>
<td>1966</td>
<td>In vitro culture is developed for protozoan and helminth parasites</td>
</tr>
<tr>
<td>1968</td>
<td>Complement fixation test for anaplasmosis is developed</td>
</tr>
<tr>
<td>1970</td>
<td>Pooled-sample technique is used to identify Trichina organisms in hog carcasses</td>
</tr>
<tr>
<td>1972</td>
<td>Parasites are identified in tissue cross-section</td>
</tr>
<tr>
<td>1972</td>
<td>Work begins on Sarcocystis fusiformis parasite of livestock</td>
</tr>
<tr>
<td>1973</td>
<td>Sheep scabies is eradicated</td>
</tr>
<tr>
<td>1978</td>
<td>First killed virus vaccine against pseudorabies in swine is licensed</td>
</tr>
<tr>
<td>1978</td>
<td>Hog cholera is finally eradicated</td>
</tr>
<tr>
<td>1981</td>
<td>A parovirus is identified as a cause of swine infertility</td>
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<tr>
<td>1982</td>
<td>A subunit vaccine against foot-and-mouth disease is developed by gene splicing</td>
</tr>
<tr>
<td>1982</td>
<td>Joint foot-and-mouth disease vaccine bands are established for the US, Canada and Mexico by tripartite agreement</td>
</tr>
<tr>
<td>1982</td>
<td>Eradication of African swine fever from Caribbean Islands is begun</td>
</tr>
</tbody>
</table>

Adapted from Dunlop and Williams (1996, Veterinary Medicine: An Illustrated History)
The emergence of antibiotics for animal use in the late 1940s transformed the veterinarians’ armoury for treatment of diseases such as mastitis. Previously, they had relied upon an array of mostly ineffective chemicals that were often highly toxic to the patient. The exceptions were the sulphonamides. The early promise of antibiotics to be the cure of all infectious diseases has not been realised despite the continued development of new generations of yet more powerful drugs. The effectiveness of many of these drugs is now waning with the ever-increasing problem of multiple antibiotic-resistant microbes developing (Johnson 1998). Antiviral drugs, although beginning to be used in human medicine, have yet to have a major impact on animal health for diseases such as bovine viral diarrhoea or infectious bovine rhinotracheitis, both of which reduce fertility.

Use of modern antihelminthics and an understanding of the epidemiology has improved health and body condition of livestock dramatically with beneficial effects upon reproductive performance. However, the increased selection pressure exerted by this intensive use of anthelmintics has led to the development of anthelmintic resistance in some parasites (Molento et al., 1999). With very few exceptions, these drugs have enabled us to control parasitic diseases but not eradicate them.

The principal of vaccination has been understood since 1798, when Jenner demonstrated that vaccination with cowpox virus effectively prevented infection with smallpox. The development of vaccines has continued at a steady pace since then. Vaccination continues to be an important defense against bacterial, viral and parasitic diseases. New vaccines against leptospirosis and bovine viral diarrhoea are effective in controlling reproductive failure caused by these infections (Dhaliwal et al., 1996). However, the early hopes that all disease could be prevented by simply injecting a susceptible animal with a killed or modified form of the causative agent has proved to be false. Even with modern technology, many major animal health problems are largely refractory to control by vaccination. Although some of the health problems that are unresponsive to vaccination are multi-factorial, conditions known to be caused by specific bacteria, such as staphylococcal mastitis, have also failed to be controlled by use of vaccination (Yancey 1999).

Ether’s properties as an anaesthetic were known as early as 1850 and the local anaesthetic was discovered Procaine in 1900. Further developments in this field led to intravenous barbiturates in the 1930s. These anaesthetics were quickly taken up by the veterinary profession and surgery performed on many conditions. Complicated orthopaedic surgery was being carried out in the 1920s and 1930s on dogs and cats, but farm animal surgery lagged well behind with caesarians on cattle not being performed regularly until the 1940s. Today, veterinary anaesthesia and surgery is probably only restricted by the cost of some of the procedures.

Agricultural practices have become increasingly more intensive over time and the public concern for farm animal welfare is reflected in the growing body of marketing organisations which guarantee certain minimum levels of care over and above what is required in legislation. Although there appears to be an increase in public disquiet over animal welfare, these concerns have been with us always, although the reasons have changed over the years. In the UK, the first legislation passed in Parliament concerning animal welfare was The Ill-Treatment of Horses Act in 1882. This possibly reflected the abundant use of the horses at that time. Further legislation followed to protect other
Table 2
British legislation concerned with animal welfare

<table>
<thead>
<tr>
<th>Legislation</th>
<th>Years</th>
</tr>
</thead>
<tbody>
<tr>
<td>The Veterinary Surgeons Acts</td>
<td>1948 and 1966</td>
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<tr>
<td>The Anaesthetics Acts</td>
<td>1954–1964</td>
</tr>
<tr>
<td>The Animals Act</td>
<td>1971</td>
</tr>
<tr>
<td>The Animal Health Act</td>
<td>1981</td>
</tr>
<tr>
<td>The Animal Health and Welfare Act</td>
<td>1984</td>
</tr>
<tr>
<td>The Animals (Scientific Procedures) Act</td>
<td>1986</td>
</tr>
</tbody>
</table>

species (Table 2) and there has been continued improvements and changes made in the legislation to cope with changing public perceptions and technological advances. Similar progress in protection of animals has occurred in most developed countries.

10. Looking to the future

Several conclusions are revealed by even a brief review of what has been achieved by animal science during the last 50 years. First, animal productivity and health are both much improved as a direct result of research. This is important for the animals as well as for us. Secondly, the manner of the contribution was not often anticipated. AI was expected to be helpful in the elimination of disease, but the great impact on genetic selection was not anticipated. By contrast, more was expected of embryo transfer than has been achieved in breed improvement. Thirdly, it has sometimes taken longer to reach a goal than was anticipated. Finally, there have also been unexpected advances. Perhaps the most striking being that for nuclear transfer from adult cells.

At present, particularly in Europe, there is a loss of public confidence in science and technology. This is seen most clearly in the response to genetically modified plants. Probably, it also overlooks and takes for granted the enormous benefits that have arisen from modern agriculture already. While it is not possible to predict what benefits will become available, we should approach the future with optimism and confidence, tempered only by concern for animal welfare, the consumer and the environment.

The session in which this paper is to be read is entitled “La main de Dieu” (The hand of God). Rather than shirk the responsibility, Homo sapiens (Man the wise) should take pride in what science continues to contribute.

References


